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(54) **Inhibitors of the advanced glycosylation of proteins and methods of use therefor.**

(57) The present invention relates to compositions and methods for inhibiting nonenzymatic cross-linking (protein aging). Accordingly, a composition is disclosed which comprises an agent capable of inhibiting the formation of advanced glycosylation endproducts of target proteins by reacting with a carbonyl moiety of an early glycosylation product of such target proteins resulting from their initial glycosylation. The method comprises contacting the target protein with the composition. Both industrial and therapeutic applications for the invention are envisioned, as food spoilage and animal protein aging can be treated.

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## RELATED PUBLICATIONS

- The Applicants are co-authors of the following articles directed to the subject matter of the present invention: "COVALENT ATTACHMENT OF SOLUBLE PROTEINS BY NONENZYMATICALLY GLYCOSYLATED COLLAGEN: ROLE IN THE IN SITU FORMATION OF IMMUNE COMPLEXES", Brownlee et al., *J. Exp. Med.*, 158, pp. 1730-1744 (1983); and "AGING OF PROTEINS: ISOLATION AND IDENTIFICATION OF FLUORESCENT CHROMOPHORE FROM THE REACTION OF POLYPEPTIDES WITH GLUCOSE", Pongor et al., *Proc. Natl. Acad. Sci. USA*, 81, pp. 2684-2688, (1984), and "ADVANCED GLYCOSYLATION ENDPRODUCTS IN TISSUE AND THE BIOCHEMICAL BASIS OF DIABETIC COMPLICATIONS", Brownlee et al., *The New Eng. J. of Med.*, 318, pp. 1315-1321 (1988). All of the above publications are incorporated herein by reference.

## BACKGROUND OF THE INVENTION

- The present invention relates generally to the aging of proteins resulting from their reaction with glucose and other reducing sugars, and more particularly to the inhibition of the reaction of nonenzymatically glycosylated proteins and the often resultant formation of advanced glycosylation endproducts and cross-links.

- The reaction between glucose and proteins has been known for some time. Its earliest manifestation was in the appearance of brown pigments during the cooking of food, which was identified by Maillard in 1912, who observed that glucose or other reducing sugars react with amino acids to form adducts that undergo a series of dehydrations and rearrangements to form stable brown pigments. Maillard, *C.R. Acad. Sci.*, 154, pp. 66-68, (1912). Further studies have suggested that stored and heat treated foods undergo nonenzymatic browning as a result of the reaction between glucose and the polypeptide chain, and that the proteins are resultingly cross-linked and correspondingly exhibit decreased bioavailability.

- This reaction between reducing sugars and food proteins was found to have its parallel *in vivo*. Thus, the nonenzymatic reaction between glucose and the free amino groups on proteins to form a stable, 1-deoxyketosyl adduct, known as the Amadori product, has been shown to occur with hemoglobin, wherein a rearrangement of the amino terminal of the beta-chain of hemoglobin by reaction with glucose, forms the adduct known as hemoglobin A<sub>1c</sub>. The reaction has also been found to occur with a variety of other body proteins, such as lens crystallins, collagen and nerve proteins. See, Bunn et al., *Biochem. Biophys. Res. Comm.*, 67, pp. 103-109 (1975); Koenig et al., *J. Biol. Chem.*, 252, pp. 2992-2997 (1977); Monnier et al., in *Maillard Reaction in Food and Nutrition*, ed. Waller, G.A., American Chemical Society, 215, pp.431-448 (1983); and Monnier and Cerami, *Clinics in Endocrinology and Metabolism*, 11, pp. 431-452 (1982).

- Moreover, brown pigments with spectral and fluorescent properties similar to those of late-stage Maillard products have also been observed *in vivo* in association with several long-lived proteins, such as lens proteins and collagen from aged individuals. An age-related linear increase in pigment was observed in human dura collagen between the ages of 20 to 90 years. See, Monnier et al., *Science*, 211, pp. 491-493 (1981); Monnier et al., *Biochem. Biophys. Acta*, 760, pp. 97-103 (1983); and, Monnier et al., *Proc. Nat. Acad. Sci.*, 81, pp. 583-587 (1984). Interestingly, the aging of collagen can be mimicked *in vitro* by the cross-linking induced by glucose; and the capture of other proteins and the formation of adducts by collagen, also noted, is theorized to occur by a cross-linking reaction, and is believed to account for the observed accumulation of albumin and antibodies in kidney basement membrane. See, Brownlee et al., *J. Exp. Med.*, 158, pp. 1739-1744 (1983); and Kohn et al., *Diabetes*, 33, No. 1, pp. 57-59 (1984).

- In Parent Application Serial No. 798,032, a method and associated agents were disclosed that served to inhibit the formation of advanced glycosylation endproducts by reacting with an early glycosylation product that results from the original reaction between the target protein and glucose. Accordingly, inhibition was postulated to take place as the reaction between the inhibitor and the early glycosylation product appeared to interrupt the subsequent reaction of the glycosylated protein with additional protein material to form the cross-linked late stage product. One of the agents identified as an inhibitor was aminoguanidine, and the results of further testing have borne out its efficacy in this regard.

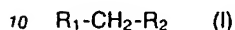
While the success that has been achieved with aminoguanidine and similar compounds is promising, a need continues to exist to identify and develop additional inhibitors that broaden the availability and perhaps the scope of this potential activity and its diagnostic and therapeutic utility.

## SUMMARY OF THE INVENTION

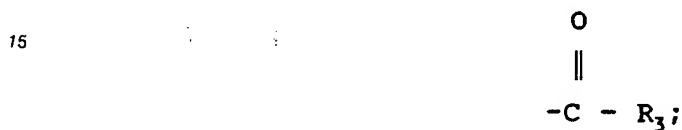
In accordance with the present invention, a method and compositions are disclosed for the inhibition of

the advanced glycosylation of proteins (protein aging). In particular, the compositions comprise agents for inhibiting nonenzymatic cross-linking (protein aging) due to the formation of advanced glycosylation endproducts. The agents may be selected from those materials capable of reacting with an early glycosylation product, including metabolic byproducts from the reaction of glucose with proteins, and thereby preventing further reactions. Cross-linking caused by other reactive sugars present *in vivo* or in foodstuffs, including ribose, galactose and fructose would also be prevented by the methods and compositions of the present invention.

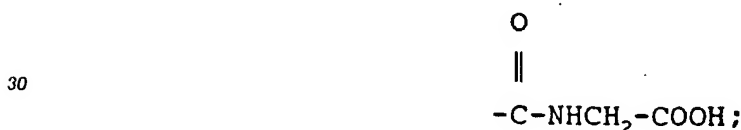
The agents comprise compounds having the following structural formula:



wherein  $R_1$  is -CN, or



20  $R_2$  is the same as  $R_1$ , or additionally can be  $-SO_2R$ ,



$R$  is a lower alkyl group;

35  $R_3$  is  $R$ ,  $-OH$ ,  $-OR$ ,  $-NH_2$ ,  $-NHNH_2$ ,  $-NHR$ ;

with the proviso that when  $R_1$  and  $R_2$  are independently selected from



wherein  $R_3$  is  $R$ ,  $-OR$  or  $-NHR$ , then the lower alkyl portion may be an alkanediyl bridge of 1-5 carbon atoms joining the molecule into a cyclic structure; their pharmaceutically acceptable acid addition salts; and a carrier therefor.

The compounds utilized in the compositions of this invention appear to react with an early glycosylation product thereby preventing the later formation of advanced glycosylation end products which lead to protein cross-links, and thereby, to protein aging.

50 The present invention also relates to a method for inhibiting protein aging by contacting an initially glycosylated protein at the stage where an early glycosylation product is present, with a quantity of one or more of the agents of the present invention, or a composition containing the same. In the instance where the present method has industrial application, one or more of the agents may be applied to the target proteins in question, either by introduction into a mixture of the same in the instance of a protein extract, or by application or introduction into foodstuffs containing the protein or proteins, all to prevent premature aging and spoilage of the particular foodstuffs.

The ability to inhibit the formation of advanced glycosylation endproducts carries with it significant implications in all applications where protein aging is a serious detriment. Thus, in the area of food

technology, the retardation of food spoilage would confer an obvious economic and social benefit by making certain foods of marginal stability less perishable and therefore more available for consumers. Spoilage would be reduced as would the expense of inspection, removal, and replacement, and the extended availability of the foods could aid in stabilizing their price in the marketplace. Similarly, in other industrial applications where the perishability of proteins is a problem, the admixture of the agents of the present invention in compositions containing such proteins would facilitate the extended useful life of the same. Presently used food preservatives and discoloration preventatives such as sulfur dioxide, known to cause toxicity including allergy and asthma in animals, can be replaced with compounds such as those described herein.

The present method has particular therapeutic application as the Maillard process acutely affects several of the significant protein masses in the body, among them collagen, elastin, lens proteins, and the kidney glomerular basement membranes. These proteins deteriorate both with age (hence the application of the term "protein aging") and a consequence of diabetes. Accordingly, the ability to either retard or substantially inhibit the formation of advanced glycosylation endproducts carries the promise of treatment for diabetes and of course, improving the quality and, perhaps, duration of animal life.

The present agents are also useful in the area of personal appearance and hygiene, as they prevent the staining of teeth by cationic anti-microbial agents with anti-plaque properties, such as chlorhexidine.

Accordingly, it is a principal object of the present invention to provide a method for inhibiting the extensive cross-linking of proteins that occurs as an ultimate consequence of the reaction of the proteins with glucose and other reactive sugars, by correspondingly inhibiting the formation of advanced glycosylation endproducts.

It is a further object of the present invention to provide a method as aforesaid which is characterized by a reaction with an initially glycosylated protein or by-product thereof, identified broadly herein as an early glycosylation product.

It is a further object of the present invention to provide a method as aforesaid which prevents the rearrangement and cross-linking of early glycosylation products to form the said advanced glycosylation endproducts.

It is a yet further object of the present invention to provide agents capable of participating in the reaction with the said early glycosylation products in the method as aforesaid.

It is a still further object of the present invention to provide therapeutic methods of treating the adverse consequences of protein aging by resort to the aforesaid method and agents.

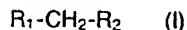
It is a still further object of the present invention to provide a method of inhibiting the discoloration of teeth by resort to the aforesaid method and agents.

It is a still further object of the present invention to provide compositions including pharmaceutical compositions, all incorporating the agents of the present invention.

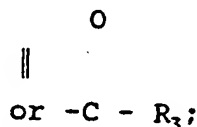
Other objects and advantages will become apparent to those skilled in the art from a consideration of the ensuing description.

#### DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

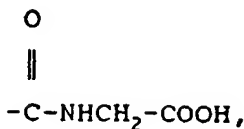
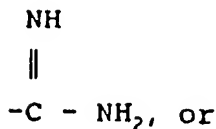
In accordance with the present invention, agents, compositions including pharmaceutical compositions containing said agents and associated methods have been developed which are believed to inhibit the formation of advanced glycosylation endproducts in a number of target proteins existing in both animals and plant material. In particular, the invention relates to a composition which may contain one or more agents comprising compounds having the structural formula



$R_1$  is -CN,



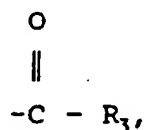
$R_2$  is the same as  $R_1$ , or additionally can be  $-SO_2R$ ,



R is a lower alkyl group;

R<sub>3</sub> is R, -OH, -OR, -NH<sub>2</sub>, -NHNH<sub>2</sub>, -NHR;

with the proviso that when R<sub>1</sub> and R<sub>2</sub> are independently selected from



wherein R<sub>3</sub> is R, -OR or -NHR, then the lower alkyl portion may be an alkanediyl bridge of 1-5 carbon atoms joining the molecule into a cyclic structure; their pharmaceutically acceptable acid addition salts; and a carrier therefor.

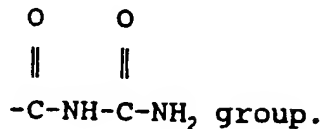
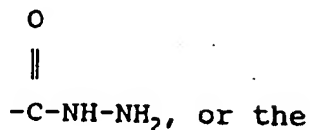
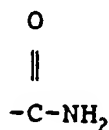
The lower alkyl and lower alkoxy groups referred to herein contain 1-6 carbon atoms and include methyl, methoxy, ethyl, ethoxy, propyl, propoxy, butyl, butoxy, pentyl, pentyloxy, hexyl, hexyloxy and the corresponding branched chain isomers thereof.

Of the compounds encompassed by Formula I, certain combinations of substituents are preferred. For

instance, when

R<sub>1</sub> is a cyano group (CN),

then R<sub>2</sub> is preferably the -SO<sub>2</sub>R,



When R<sub>1</sub> is a



with  $\text{R}_3$  being an amino group, then  $\text{R}_2$  is preferably a



20 When  $\text{R}_1$  is a



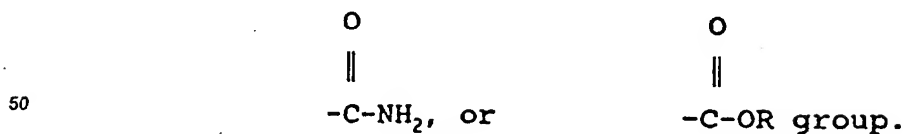
30 with  $\text{R}_3$  being a hydroxy group, then  $\text{R}_2$  is preferably a



When  $\text{R}_1$  is a

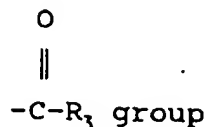


45 with  $\text{R}_3$  being a hydroxy group, then  $\text{R}_2$  is preferably a



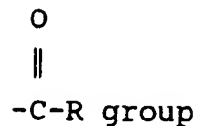
When  $\text{R}_1$  is a

55



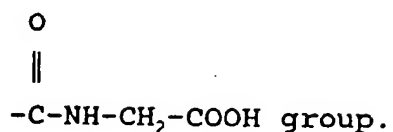
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with  $\text{R}_3$  being R, then  $\text{R}_2$  is preferably a



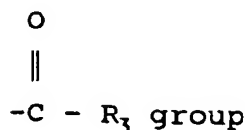
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15 or a



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When  $\text{R}_1$  is a

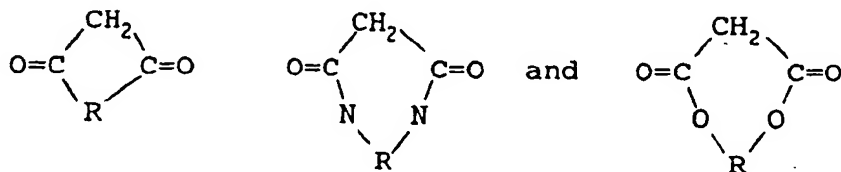


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with  $\text{R}_3$  being an -R, -NHR or OR group, then a highly preferred group is that where the  $\text{R}_2$  is identical to  $\text{R}_1$  and the alkyl group of R forms an alkanediyl bridge between the two sides of the molecule, i.e., compounds of the formulae

35



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In these formulae, R is preferably an alkanediyl group of 1-5 carbon atoms.  
(isopropylidene malonate);

45 5, 5-dimethyl-1,3-cyclohexanedione (dimedone);

1,3-cyclohexanedione;

1,3-cyclopentanedione;

tetrahydrofuran-2,4-dione (tetronic acid);

2,4-pyrrolidinedione (tetramic acid);

50 1-methyl-2,4-pyrrolidinedione;

5-sec-butyl-2,4-pyrrolidinedione;

cyanoacetamide;

N-methylacetoacetamide;

cyanoacetylurea;

55 acetoacetylglycine;

malonic acid dihydrazide;

2,4-pentanedione (acetylacetone);

3-oxobutanamide;

methyl 4,4-dimethyl-3-oxopentanoate;  
 malonic acid;  
 monomethyl potassium malonate (malonic acid monomethyl ester potassium salt);  
 and

5 monoethyl hydrogen malonate.

The above compounds are capable of inhibiting the formation of advanced glycosylation endproducts on target proteins. The cross-linking of the protein to form the advanced glycosylation endproduct contributes to the entrapment of other proteins and results in the development *in vivo* of conditions such as reduced elasticity and wrinkling of the skin, certain kidney diseases, atherosclerosis, osteoarthritis and the

10 like. Similarly, plant material that undergoes nonenzymatic browning deteriorates and, in the case of foodstuffs, become spoiled or toughened and, consequently, inedible. Thus, the compounds employed in accordance with this invention inhibit this late stage Maillard effect and intervene in the deleterious changes described above.

The rationale of the present invention is to use agents which block the post-glycosylation step, i.e., the

15 formation of fluorescent chromophores such as that identified in Pongor, et al., *supra* and Farmer et al., *supra*, among others, the presence of which chromophores is associated with, and leads to adverse sequelae of diabetes and aging. An ideal agent would prevent the formation of the chromophore and its associate cross-links of proteins to proteins and trapping of proteins on the other proteins, such as occurs in arteries and in the kidney.

20 The chemical nature of the early glycosylation products with which the compounds of the present invention are believed to react may vary, and accordingly the term "early glycosylation product(s)" as used herein is intended to include any and all such variations within its scope. For example, early glycosylation products with carbonyl moieties that are involved in the formation of advanced glycosylation endproducts, and that may be blocked by reaction with the compounds of the present invention, have been postulated. In

25 one embodiment, it is envisioned that the early glycosylation product may comprise the reactive carbonyl moieties of Amadori products or their further condensation, dehydration and/or rearrangement products, which may condense to form advanced glycosylation endproducts. In another scenario, reactive carbonyl compounds, containing one or more carbonyl moieties (such as glycolaldehyde, glyceraldehyde or 3-deoxyglucosone) may form from the cleavage of Amadori or other early glycosylation endproducts (see, for example, Gottschalk, A. (1972) in *The Glycoproteins* (Gottschalk, A., ed) Part A, pp. 141-157, Elsevier Publishing Co., New York; Reynolds, T.M. (1965) *Adv. Food Res.*, 14, pp. 167-283), and by subsequent

30 reactions with an amine or Amadori product, may form carbonyl-containing advanced glycosylation products such as the alkylformyl-glycosylpyrroles described by Farmer et al., *supra*.

Several investigators have studied the mechanism of advanced glycosylation product formation. *In vitro* studies by Eble et al., (1983), "Nonenzymatic Glucosylation and Glucose-dependent Cross-linking of Protein", *J. Biol. Chem.*, 258:9406-9412, concerned the cross-linking of glycosylated protein with non-glycosylated protein in the absence of glucose. Eble et al. sought to elucidate the mechanism of the Maillard reaction and accordingly conducted controlled initial glycosylation of RNAase as a model system, which was then examined under varying conditions. In one aspect, the glycosylated protein material was

40 isolated and placed in a glucose-free environment and thereby observed to determine the extent of cross-linking.

Eble et al. thereby observed that cross-linking continued to occur not only with the glycosylated protein but with non-glycosylated proteins as well. One of the observations noted by Eble et al. was that the reaction between glycosylated protein and the protein material appeared to occur at the location on the

45 protein chain of the amino acid lysine. Confirmatory experimentation conducted by Eble et al. in this connection demonstrated that free lysine would compete with the lysine on RNAase for the binding of glycosylated protein. Thus, it might be inferred from these data that lysine may serve as an inhibitor of advanced glycosylation; however, this conclusion and the underlying observations leading to it should be taken in the relatively limited context of the model system prepared and examined by Eble et al. Clearly,

50 Eble et al. does not appreciate, nor is there a suggestion therein, of the discoveries that underlie the present invention, with respect to the inhibition of advanced glycosylation of proteins both *in vitro* and *in vivo*.

The experiments of Eble et al. do not suggest the reactive cleavage product mechanism or any other mechanism in the *in vivo* formation of advanced glycosylation endproducts in which glucose is always

55 present. In fact, other investigators support this mechanism to explain the formation of advanced glycosylated endproducts *in vivo* (see for example Hayase et al, 1989, *supra*; Sell and Monnier, 1989, *supra*; Oimomi et al., *Agric. Biol. Chem.*, 53(6):1727-1728 (1989); and *Diabetes Research and Clinical Practice*, 6:311-313 (1989). Accordingly, the use of lysine as an inhibitor in the Eble et al. model system has no bearing upon



the utility of the compounds of the present invention in the inhibition of advanced glycosylated endproduct formation in the presence of glucose in vivo, and the amelioration of complications of diabetes and aging.

The compositions useful in the present invention comprise or contain agents capable of reacting with the active carbonyl intermediate of the early glycosylation product as defined herein. Suitable agents are  
5 the compounds of Formula I of the present invention.

The present invention likewise relates to methods for inhibiting the formation of advanced glycosylation endproducts, which comprise contacting the target proteins with a composition of the present invention. In the instance where the target proteins are contained in foodstuffs, whether of plant or animal origin, these foodstuffs could have applied to them by various conventional means a composition containing the present  
10 agents.

In the food industry, sulfites were found years ago to inhibit the Maillard reaction and are commonly used in processed and stored foods. Recently, however, sulfites in food have been implicated in severe and even fatal reactions in asthmatics. As a consequence, the sulfite treatment of fresh fruits and vegetables has been banned. The mechanism for the allergic reaction is not known. Accordingly, the present compositions  
15 and agents offer a nontoxic alternative to sulfites in the treatment of foods in this manner.

As is apparent from a discussion of the environment of the present invention, the present methods and compositions hold the promise for arresting the aging of key proteins both in animals and plants, and concomitantly, conferring both economic and medical benefits as a result thereof. In the instance of foodstuffs, the administration of the present composition holds the promise for retarding food spoilage  
20 thereby making foodstuffs of increased shelf life and greater availability to consumers. Replacement of currently-used preservatives, such as sulfur dioxide known to cause allergies and asthma in humans, with non-toxic, biocompatible compounds is a further advantage of the present invention.

The therapeutic implications of the present invention relate to the arrest of the aging process which has, as indicated earlier, been identified in the aging of key proteins by advanced glycosylation and cross-  
25 linking. Thus, body proteins, and particularly structural body proteins, such as collagen, elastin, lens proteins, nerve proteins, kidney glomerular basement membranes and other extravascular matrix components would all benefit in their longevity and operation from the practice of the present invention. The present invention thus reduces the incidence of pathologies involving the entrapment of proteins by cross-linked target proteins, such as retinopathy, cataracts, diabetic kidney disease, glomerulosclerosis, peripheral  
30 vascular disease, arteriosclerosis obliterans, peripheral neuropathy, stroke, hypertension, atherosclerosis, osteoarthritis, periarticular rigidity, loss of elasticity and wrinkling of skin, stiffening of joints, glomerulonephritis, etc. Likewise, all of these conditions are in evidence in patients afflicted with diabetes mellitus. Thus, the present therapeutic method is relevant to treatment of the noted conditions in patients either of advanced age or those suffering from one of the mentioned pathologies.

Protein cross-linking through advanced glycosylation product formation can decrease solubility of structural proteins such as collagen in vessel walls (see Brownlee et al., Science, 232, pp. 1629-1632, (1986)), and can also trap serum proteins, such as lipoproteins to the collagen. Also, this may result in increased permeability of the endothelium and consequently covalent trapping of extravasated plasma proteins in subendothelial matrix, and reduction in susceptibility of both plasma and matrix proteins to  
40 physiologic degradation by enzymes. (See Brownlee et al., Diabetes, 35, Suppl. 1, p. 42A (1986)). For these reasons, the progressive occlusion of diabetic vessels induced by chronic hyperglycemia has been hypothesized to result from excessive formation of glucose-derived cross-links. Such diabetic macrovascular changes and microvascular occlusion can be effectively prevented by chemical inhibition of advanced glycosylation product formation utilizing a composition and the methods of the present invention.

Studies indicate that the development of chronic diabetic damage in target organs is primarily linked to hyperglycemia so that tight metabolic control would delay or even prevent end-organ damage. See Nicholls et al., Lab. Invest., 60, No. 4, p. 486 (1989), which discusses the effects of islet isografting and aminoguanidine in murine diabetic nephropathy. These studies further evidence that aminoguanidine diminishes aortic wall protein cross-linking in diabetic rats and confirm earlier studies by Brownlee et al.,  
50 Science, 232, pp. 1629-1632 (1986) to this additional target organ of complication of diabetes. Also, an additional study showed the reduction of immunoglobulin trapping in the kidney by aminoguanidine (Brownlee et al., Diabetes, 35, Suppl. 1, p. 42A (1986)).

Further evidence in the streptozotocin-diabetic rat model that aminoguanidine administration intervenes in the development of diabetic nephropathy was presented by Brownlee et al., 1988, supra, with regard to  
55 morphologic changes in the kidney which are hallmarks of diabetic renal disease. These investigators reported that the increased glomerular basement membrane thickness, a major structural abnormality characteristic of diabetic renal disease, was prevented with aminoguanidine.

Taken together, these data strongly suggest that inhibition of the formation of advanced glycosylation

endproducts (AGEs), by the teaching of the present invention, may prevent late, as well as early, structural lesions due to diabetes, as well as changes during aging caused by the formation of AGE's.

Diabetes-induced changes in the deformability of red blood cells, leading to more rigid cell membranes, is another manifestation of cross-linking and aminoguanidine has been shown to prevent it *in vivo*. In such studies, New Zealand White rabbits, with induced, long-term diabetes are used to study the effects of a test compound on red blood cell (RBC) deformability (df). The test compound is administered at a rate of 100 mg/kg by oral gavage to diabetic rabbits (Brown et al., Presentation of Abstract for Association for Academic Minority Physicians, Annual Scientific Meeting (1989)).

Increased cross-linking of collagen in diabetic rats has shown to be prevented by aminoguanidine. Oxlund and Andreassen, "The increase in biochemical and biomechanical stability of collagen in diabetic rats is prevented by aminoguanidine treatment", European Association for the Study of Diabetes, Twenty-fifth Annual Meeting, p. 525A, Abstract No. 371, 1989 showed the effect when thermal stability of tendon fibers was assessed by breaking time in a urea bath, as well as mechanical strength. Soulis et al., "Aminoguanidine reduces tissue fluorescence but not albuminuria in diabetic rats". NIH Conference on the Maillard Reaction in Aging, Diabetes, and Nutrition, Bethesda, Maryland, September 22-23, 1988, page 30) showed the same effect on collagen in the aorta, measured by fluorescence and solubility.

Giambone and Brownlee, "Aminoguanidine Treatment Normalizes Increased Steady-state Levels of Laminin B1 mRNA in Kidneys of Long-term Streptozotocin-diabetic Rats" *Diabetes*, 38, Supplement 2:83A Forty-ninth Annual Meeting, American Diabetes Association (1989) showed that aminoguanidine treatment to diabetic rats prevents the diabetes-induced increase in laminin B<sub>1</sub> mRNA in the kidney. This indicates that aminoguanidine may prevent overproduction of matrix, which leads to basement membrane thickening and morphologic and functional deterioration of vasculature in kidneys and other organs.

A further consequence of diabetes is the hyperglycemia-induced matrix bone differentiation resulting in decreased bone formation usually associated with chronic diabetes. In animal models, diabetes reduces matrix-induced bone differentiation by 70% (*Am. J. Phys.*, 238 (1980)).

In the instance where the compositions of the present invention are utilized for *in vivo* or therapeutic purposes, it may be noted that the compounds or agents used therein are biocompatible. Pharmaceutical compositions may be prepared with a therapeutically effective quantity of the agents or compounds of the present invention and may include a pharmaceutically acceptable carrier, selected from known materials utilized for this purpose. Such compositions may be prepared in a variety of forms, depending on the method of administration. Also, various pharmaceutically acceptable addition salts of the compounds of Formula I may be utilized.

A liquid form would be utilized in the instance where administration is by intravenous, intramuscular or intraperitoneal injection. When appropriate, solid dosage forms such as tablets, capsules, or liquid dosage formulations such as solutions and suspensions, etc., may be prepared for oral administration. For topical or dermal application to the skin or eye, a solution, a lotion or ointment may be formulated with the agent in a suitable vehicle such as water, ethanol, propylene glycol, perhaps including a carrier to aid in penetration into the skin or eye. For example, a topical preparation could include up to about 10% of the compound of Formula I. Other suitable forms for administration to other body tissues are also contemplated.

In the instance where the present method has therapeutic application, the animal host intended for treatment may have administered to it a quantity of one or more of the agents, in a suitable pharmaceutical form. Administration may be accomplished by known techniques, such as oral, topical and parenteral techniques such as intradermal, subcutaneous, intravenous or intraperitoneal injection, as well as by other conventional means. Administration of the agents may take place over an extended period of time at a dosage level of, for example, up to about 25 mg/kg.

As noted earlier, the invention also extends to a method of inhibiting the discoloration of teeth resulting from nonenzymatic browning in the oral cavity which comprises administration to a subject in need of such therapy an amount effective to inhibit the formation of advanced glycosylation endproducts of a composition comprising an agent of structural Formula I.

The nonenzymatic browning reaction which occurs in the oral cavity results in the discoloration of teeth. Presently used anti-plaque agents accelerate this nonenzymatic browning reaction and further the staining of the teeth. Recently, a class of cationic anti-microbial agents with remarkable anti-plaque properties have been formulated in oral rinses for regular use to kill bacteria in the mouth. These agents, the cationic antiseptics, include such agents as alexidine, cetyl pyridinium chloride, chlorhexidine gluconate, hexetidine, and benzalkonium chloride.

Tooth staining by chlorhexidine and other anti-plaque agents apparently results from the enhancement of the Maillard reaction. Nordbo, *J. Dent. Res.*, 58, p. 1429 (1979) reported that chlorhexidine and benzalkonium chloride catalyze browning reactions *in vitro*. Chlorhexidine added to mixtures containing a

sugar derivative and a source of amino groups underwent increased color formation, attributed to the Maillard reaction. It is also known that use of chlorhexidine results in an increased dental pellicle. Nordbo proposed that chlorhexidine resulted in tooth staining in two ways: first, by increasing formation of pellicle which contains more amino groups, and secondly, by catalysis of the Maillard reaction leading to colored products.

In accordance with this method, the compounds of Formula I are formulated into compositions adapted for use in the oral cavity. Particularly suitable formulations are oral rinses and toothpastes incorporating the active agent.

In the practice of this invention, conventional formulating techniques are utilized with nontoxic, pharmaceutically acceptable carriers typically utilized in the amounts and combinations that are well-known for the formulation of such oral rinses and toothpastes.

The agent of Formula I is formulated in compositions in an amount effective to inhibit the formation of advanced glycosylation endproducts. This amount will, of course, vary with the particular agent being utilized and the particular dosage form, but typically is in the range of 0.01% to 1.0%, by weight, of the particular formulation.

Additionally, since the agents of the aforesaid method are concentrated in the salivary glands upon oral ingestion or parenteral administration, they can be so administered. This concentration in the salivary glands results in their secretion into saliva, the net result being that they are functionally placed in the oral cavity where they can effect their desired method. For such administration, the particular agent can be formulated in any conventional oral or parenteral dosage form. A particularly desirable dosage form is the incorporation of the agent into a vitamin tablet or fluoride tablet so as to maximize patient, and particularly juvenile patient, compliance.

The compounds encompassed by Formula I are conveniently prepared by chemical syntheses well-known in the art. Certain of the compounds encompassed by Formula I are well-known compounds readily available from chemical supply houses and/or preparable by synthetic methods specifically published therefor. For instance, the following compounds are available from Aldrich Chemical Company (Milwaukee, Wisconsin):

propanediamide (malonamide);  
3-amino-3-iminopropanamide monohydrochloride (malonamamide HCl);  
cyanoacetic acid hydrazide (cyanoacetohydrazide, cyacetacide);  
methylsulfonylacetonitrile;  
2,2-dimethyl-1,3-dioxane-4,6-dione (isopropylidene malonate);  
5,5-dimethyl-1,3-cyclohexanedione (dimedone);  
cyanoacetamide;  
cyanoacetylurea;  
acetoacetyl glycine;  
malonic acid dihydrazide;  
2,4-pentanedione;  
3-oxobutanamide;  
malonic acid; and  
monomethyl potassium malonate (malonic acid monomethyl ester potassium salt).

Other compounds described in the chemical and patent literature and encompassed by Formula I are those such as

cyanoacetic acid;  
methanesulfonylacetone;  
methanesulfonyl acetamide;  
diethyl malonate;  
dimethyl malonate;  
ethyl methanesulfonylacetate;  
malononitrile;  
methyl 4,4-dimethyl-3-oxopentanoate;  
4,4-dimethyl-3-oxopentanitrile;  
N-methylacetoacetamide;  
monoethyl hydrogen malonate;  
1,3-cyclohexanedione;  
1,3-cyclopentanedione;  
tetrahydrofuran-2,4-dione (tetronic acid);  
2,4-pyrrolidinedione (tetramic acid);

1-methyl-2,4-pyrrolidinedione [Lee et al., J. Am. Chem. Soc. 100:4225-4236 (1978)]; and  
 5-sec-butyl-2,4-pyrrolidinedione [Stickings et al., Biochem. J., 78:412-418 (1961)].

The present invention will be better understood from a review of the following illustrative examples.

#### 5 EXAMPLE 1

The following method was used to evaluate the ability of the compounds of the present invention to inhibit glucose-mediated development of fluorescence of bovine serum albumin (BSA), a measure of cross-linking. Compounds were incubated under aseptic conditions at a concentration of 1 mM with 400 mM  
 10 glucose and 100 mg/mL BSA in a 1.5 M sodium phosphate buffer, pH 7.4.

Samples of the incubation mixture were taken immediately and after 1 week incubation at 37° C for measurement of fluorescence. For each test compound, control incubations in buffer were made of compound alone (C), compound plus glucose (G + C), and compound plus BSA (B + C). An additional set of incubations of glucose and BSA (B + G) were prepared as the baseline controls against which were  
 15 measured the ability of the compounds to inhibit. Each incubation was made in triplicate.

Fluorescence (excitation, 370 nm; emission, 440 nm) was measured on each sample after a 100-fold dilution in distilled water.

The % inhibition of browning of each test compound was calculated as follows. Each F represents the fluorescence measurement of that sample after 1 week incubation less its fluorescence before incubation.

20

% inhibition =

$$\frac{F_{B+G} - [F_{B+G+C} - (F_C + F_{G+C} + F_{B+C})]}{F_{B+G}} \times 100$$

25

where B = BSA, G = glucose, and C = test compound.

Percent inhibition of browning by various test compounds at 1 mM:

30	0%	no inhibitor;
	56.9%	propanediamide (malonamide);
	51.9%	3-amino-3-iminopropanamide monohydrochloride (malonamamide HCl);
	51.9%	cyanoacetic acid hydrazide (cyanoacetohydrazide, cyacetacide);
	35.8%	methylsulfonylacetonitrile;
35	24.1%	2,2-dimethyl-1,3-dioxane-4,6-dione (isopropylidene malonate; Meldrum's acid);
	47.1%	5,5-dimethyl-1,3-cyclohexanedione (dimedone);
	35.1%	acetoacetyl glycine;
	69.9%	malonic acid dihydrazide;
	51.6%	2,4-pentanedione;
40	33.2%	3-oxobutanamide;
	40.6%	malonic acid;
	35.8%	monomethyl potassium malonate (malonic acid monomethyl ester potassium salt).

The above experiments suggest that this type of drug therapy may have benefit in reducing the pathology associated with the advanced glycosylation of proteins and the formation of cross-links between  
 45 proteins and other macromolecules. Drug therapy may be used to prevent the increased trapping and cross-linking of proteins that occurs in diabetes and aging which leads to sequelae such as retinal damage, and extra-vascularly, damage to tendons, ligaments and other joints. This therapy might retard atherosclerosis and connective tissue changes that occur with diabetes and aging. Both topical, oral, and parenteral routes of administration to provide therapy locally and systemically are contemplated.

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#### EXAMPLE 2

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	<u>Tablet</u>	<u>mg/tablet</u>
	Compound of Formula I	50
5	Starch	50
	Mannitol	75
	Magnesium stearate	2
10	Stearic acid	5

The compound, a portion of the starch and the lactose are combined and wet granulated with starch paste. The wet granulation is placed on trays and allowed to dry overnight at a temperature of 45° C. The dried granulation is comminuted in a comminutor to a particle size of approximately 20 mesh. Magnesium stearate, stearic acid and the balance of the starch are added and the entire mix blended prior to compression on a suitable tablet press. The tablets are compressed at a weight of 232 mg. using a 11/32" punch with a hardness of 4 kg. These tablets will disintegrate within a half hour according to the method described in USP XVI.

#### 20 EXAMPLE 3

	<u>Lotion</u>	<u>mg/g</u>
25	Compound of Formula I	1.0
	Ethyl alcohol	400.0
	Polyethylene glycol 400	300.0
30	Hydroxypropyl cellulose	5.0
	Propylene glycol	to make 1.0 g

#### 35 EXAMPLE 4

	<u>Oral Rinse</u>	
40	Compound of Formula I:	1.4 %
	Chlorhexidine gluconate	0.12 %
	Ethanol	11.6 %
	Sodium saccharin	0.15 %
45		
	FD&C Blue No. 1	0.001%
50	Peppermint Oil	0.5 %
	Glycerine	10.0 %
	Tween 60	0.3 %
55	Water to	100 %

#### EXAMPLE 5

Toothpaste

	Compound of Formula I:	5.5	%
5	Sorbitol, 70% in water	25	%
	Sodium saccharin	0.15	%
	Sodium lauryl sulfate	1.75	%
10	Carbopol 934, 6% dispersion in water	15	%
	Oil of Spearmint	1.0	%
	Sodium hydroxide, 50% in water	0.76	%
	Dibasic calcium phosphate dihydrate	45	%
15	Water to	100	%

EXAMPLE 6

20

To further study the ability of inhibitors of nonenzymatic browning to prevent the discoloration of protein on a surface, such as that which occurs on the tooth surface, the following surface browning experiment is performed. As a substitute for a pellicle-covered tooth surface, unexposed and developed photographic paper is used to provide a fixed protein (gelatin, i.e., collagen) surface on a paper backing. Five millimeter circles are punched and immersed for one week at 50 °C in a solution of 100 mM glucose-6-phosphate in a 0.5 M phosphate buffer, pH 7.4, containing 3 mM sodium azide. Glucose-6-phosphate is a sugar capable of participating in nonenzymatic browning at a more rapid rate than glucose. In addition to the glucose-6-phosphate, chlorhexidine and/or a compound of Formula I are included. After incubation, the gelatin/paper disks are rinsed with water, observed for brown color, and photographed.

30 Incubation of the disks in glucose-6-phosphate alone shows slight brown color versus disks soaked in buffer alone. Inclusion of chlorhexidine (in the form of Peridex® at a final concentration of 0.04% chlorhexidine) shows significant browning. Addition of a compound of Formula I to the chlorhexidine completely inhibits browning of the gelatin, as does inclusion of a compound of Formula I in the absence of chlorhexidine.

35 The slight brown color formed by the action of glucose-6-phosphate on the gelatin surface alone and its prevention by a compound of Formula I demonstrates the utility of the present invention in preventing nonenzymatic browning of tooth surfaces. The enhanced browning in the presence of chlorhexidine and its prevention with a compound of Formula I demonstrates the utility of the present invention in preventing the anti-plaque agent-enhanced nonenzymatic browning which occurs with chlorhexidine.

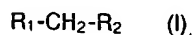
40 This invention may be embodied in other forms or carried out in other ways without departing from the spirit or essential characteristics thereof. The present disclosure is therefore to be considered as in all respects illustrative and not restrictive, the scope of the invention being indicated by the appended Claims, and all changes which come within the meaning and range of equivalency are intended to be embraced therein.

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Claims

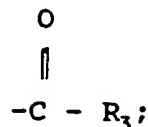
1. A composition for inhibiting the advanced glycosylation of a target protein comprising an effective amount of a compound selected from the group consisting of compounds of the formula

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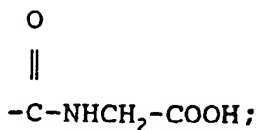
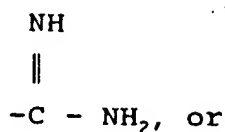
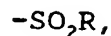
wherein

55  $R_1$  is -CN, or



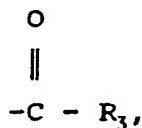
$\text{R}_3$  is R, -OH, -OR, -NH<sub>2</sub>, -NHNH<sub>2</sub>, -NHR;

$\text{R}_2$  is the same as  $\text{R}_1$ , or additionally can be



R is a lower alkyl group;

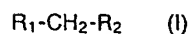
with the proviso that when  $\text{R}_1$  and  $\text{R}_2$  are independently selected from



wherein  $\text{R}_3$  is R, -OR or -NHR, then the lower alkyl portion may be an alkanediyl bridge of 1-5 carbon atoms joining the molecule into a cyclic structure; their biocompatible acid addition salts, and mixtures thereof; and

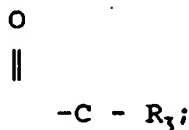
a carrier therefor.

2. A pharmaceutical composition for administration to an animal to inhibit the advanced glycosylation of a target protein within said animal, comprising a pharmaceutically effective amount of a compound selected from the group consisting of compounds of the formula

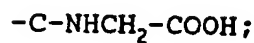
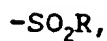


wherein

$\text{R}_1$  is -CN,



or  $\text{R}_2$  is the same as  $\text{R}_1$ , or additionally can be



R is a lower alkyl group;

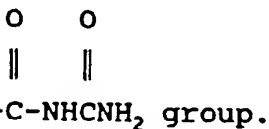
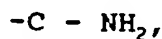
R<sub>3</sub> is R, -OH, -OR, -NH<sub>2</sub>, -NHNH<sub>2</sub>, -NHR;

with the proviso that when R<sub>1</sub> and R<sub>2</sub> are independently selected from



wherein R<sub>3</sub> is R, -OR or -NHR, then the lower alkyl portion may be an alkanediyl bridge of 1-5 carbon atoms joining the molecule into a cyclic structure; their pharmaceutically acceptable acid addition salts; and mixtures thereof; and a pharmaceutically acceptable carrier therefor.

3. The composition of Claims 1 or 2 wherein said compound has the formula wherein R<sub>1</sub> is a cyano group and R<sub>2</sub> is a -SO<sub>2</sub>R,



4. The composition of Claims 1 or 2 wherein said compound is methylsulfonylacetoneitrile.

5. The composition of Claims 1 or 2 wherein said compound is cyanoacetamide.

6. The composition of Claims 1 or 2 wherein said compound is cyanoacetic acid hydrazide.



7. The composition of Claims 1 or 2 wherein said compound is cyanoacetylurea.

8. The composition of Claims 1 or 2 wherein said compound has the formula wherein  $R_1$  is a



10 with  $R_3$  being an amino group and  $R_2$  is a group identical to  $R_1$  or a



20 9. The composition of Claims 1 or 2 wherein said compound is propanediamide.

10. The composition of Claims 1 or 2 wherein said compound is 3-amino-3-iminopropanamide monohydrochloride.

25 11. The composition of Claims 1 or 2 wherein said compound has the formula wherein  $R_1$  is a



with  $R_3$  being a hydroxy amino group and  $R_2$  is a group identical to  $R_1$  or a



40

12. The composition of Claims 1 or 2 wherein said compound is malonic acid.

13. The composition of Claims 1 or 2 wherein said compound is monomethyl potassium malonate.

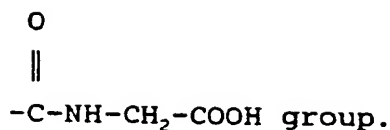
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14. The composition of Claims 1 or 2 wherein said compound has the formula wherein  $R_1$  is a

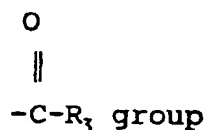


with  $R_3$  being R, and  $R_2$  is a group identical to  $R_1$  or a

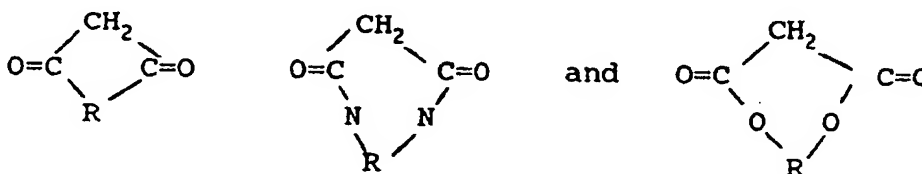
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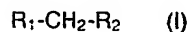
15. The composition of Claims 1 or 2 wherein said compound is 2,4-pentanedione.
16. The composition of Claims 1 or 2 wherein said compound is acetoacetyl glycine.
17. The composition of Claims 1 or 2 wherein said compound has the formula wherein  $R_1$  is a



with  $R_3$  being a -R, -NHR or -OR group,  $R_2$  being identical to  $R_1$  and the alkyl group of R forms an alkanediyl bridge between the two sides of the molecule, forming compounds of the formula

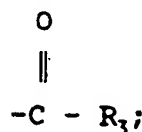


18. The composition of Claim 17 wherein R contains 1-5 carbon atoms.
19. The composition of Claims 1 or 2 wherein said compound is 5,5-dimethyl-1,3-cyclohexanedione.
20. The composition of Claims 1 or 2 wherein said compound is malonic acid dihydrazide.
21. The composition of Claims 1 or 2 wherein said compound is methylsulfonylacetonitrile.
22. A method for inhibiting the advanced glycosylation of a target protein comprising contacting the target protein with an effective amount of composition comprising a compound selected from the group consisting of compounds of the formula

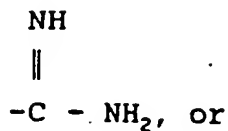


wherein

$R_1$  is -CN, or



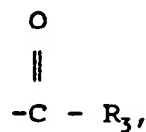
$R_2$  is the same as  $R_1$ , or additionally can be  $-\text{SO}_2\text{R}$ ,



R is a lower alkyl group;

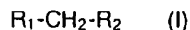
R<sub>3</sub> is R, -OH, -OR, -NH<sub>2</sub>, -NHNH<sub>2</sub>, -NHR;

with the proviso that when R<sub>1</sub> and R<sub>2</sub> are independently selected from



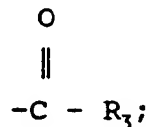
wherein R<sub>3</sub> is R, -OR or -NR, then the lower alkyl portion may be an alkanediyl bridge of 1-5 carbon atoms joining the molecule into a cyclic structure; their pharmaceutically acceptable acid addition salts, and mixtures thereof; and  
a carrier therefor.

23. A method for treating an animal to inhibit the formation of advanced glycosylation endproducts of a target protein within said animal, said method comprising administering an effective amount of a pharmaceutical composition, said pharmaceutical composition comprising a compound selected from the group consisting of compounds of the formula



wherein

R<sub>1</sub> is -CN, or



R<sub>2</sub> is the same as R<sub>1</sub>, or additionally can be -SO<sub>2</sub>R,



R is a lower alkyl group;

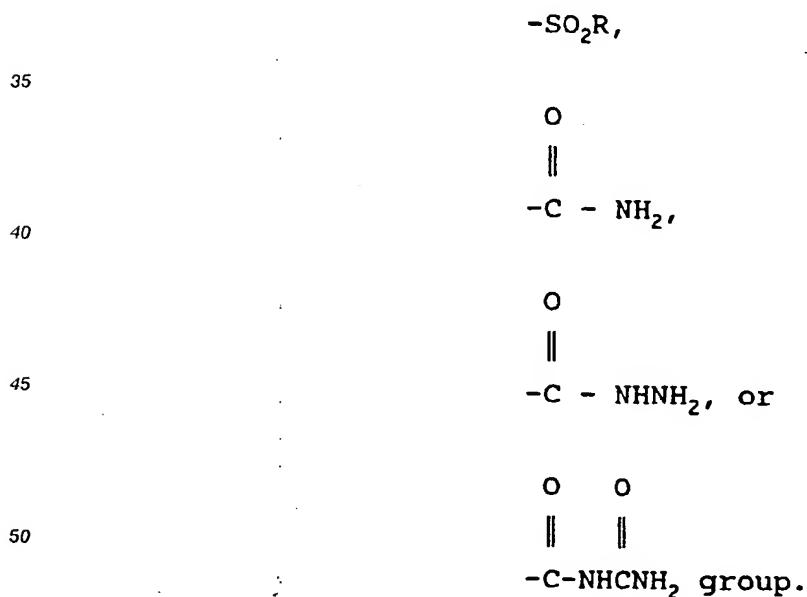
15  $R_3$  is R, -OH, -OR, -NH<sub>2</sub>, -NHNH<sub>2</sub>, -NHR;

with the proviso that when  $R_1$  and  $R_2$  are independently selected from



25 wherein  $R_3$  is R, -OR or -NHR, then the lower alkyl portion may be an alkanediyl bridge of 1-5 carbon atoms joining the molecule into a cyclic structure; their pharmaceutically acceptable acid addition salts and mixtures thereof; and  
a pharmaceutically acceptable carrier therefor.

30 24. The method of Claim 23 wherein said compound has the formula wherein  $R_1$  is a cyano group and  $R_2$  is a



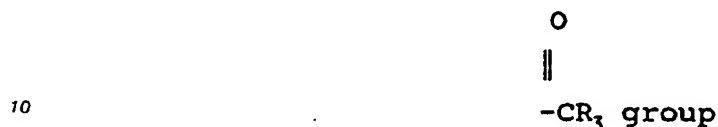
55 25. The method of Claim 23 wherein said compound is methylsulfonylacetonitrile.

26. The method of Claim 23 wherein said compound is cyanoacetamide.

27. The method of Claim 23 wherein said compound is cyanoacetic acid hydrazide.

28. The method of Claim 23 wherein said compound is cyanoacetylurea.

5 29. The method of Claim 23 wherein said compound has the formula wherein  $R_1$  is a



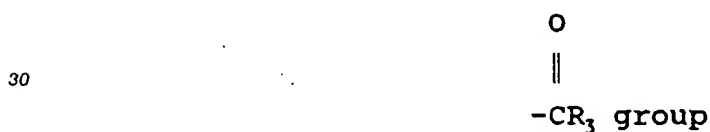
with  $R_3$  being an amino group and  $R_2$  is a group identical to  $R_1$  or a



30. The method of Claim 23 wherein said compound is propanediamide.

31. The method of Claim 23 wherein said compound is 3-amino-3-iminopropanamide monohydrochloride.

25 32. The method of Claim 23 wherein said compound has the formula wherein  $R_1$  is a



with  $R_3$  being a hydroxy amino group and  $R_2$  is a group identical to  $R_1$  or a



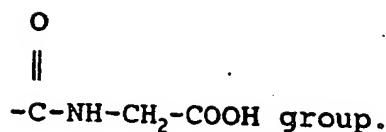
33. The method of Claim 23 wherein said compound is malonic acid.

45 34. The method of Claim 23 wherein said compound is monomethyl potassium malonate.

35. The method of Claim 23 wherein said compound has the formula wherein  $R_1$  is a



55 with  $R_3$  being  $R_1$  and  $R_2$  is a group identical to  $R_1$  or a



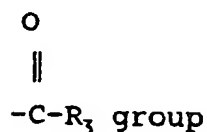
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36. The method of Claim 23 wherein said compound is 2,4-pentanedione.

10 37. The method of Claim 23 wherein said compound is acetoacetyl glycine.

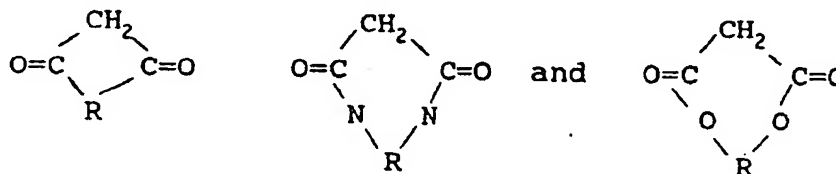
38. The method of Claim 23 wherein said compound has the formula wherein  $R_1$  is a

15



20 with  $R_3$  being a -R, -NHR or -OR group,  $R_2$  being identical to  $R_1$  and the alkyl group of R forms an alkanediyl bridge between the two sides of the molecule, forming compounds of the formula

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39. The method of Claim 23 wherein R contains 2-5 carbon atoms.

40. The method of Claim 23 wherein said compound is 5,5-dimethyl-1,3-cyclohexanedione.

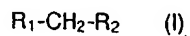
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41. The method of Claim 23 wherein said compound is malonic acid dihydrazide.

42. The method of Claim 23 wherein said compound is methylsulfonylacetonitrile.

40 43. A method of inhibiting the discoloration of teeth resulting from non-enzymatic browning in the oral cavity which comprises administration of an amount effective to inhibit the formation of advanced glycosylation endproducts of a composition comprising a compound selected from the group consisting of compounds of the formula

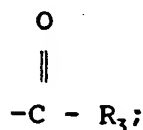
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wherein R is a lower alkyl group;

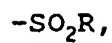
$R_1$  is -CN, or

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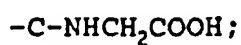
$R_2$  is the same as  $R_1$ , or additionally can be



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$\text{R}_3$  is R, -OH, -OR, -NH<sub>2</sub>, -NHNH<sub>2</sub>, -NHR;

with the proviso that when  $\text{R}_1$  and  $\text{R}_2$  are independently selected from

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wherein  $\text{R}_3$  is R, -OR or -NHR, then the lower alkyl portion may be an alkanediyl bridge of 1-5 carbon atoms joining the molecule into a cyclic structure; their pharmaceutically acceptable acid addition salts and mixtures thereof; and

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a pharmaceutically acceptable carrier therefor.

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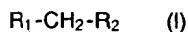
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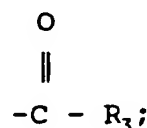
(54) **Inhibitors of the advanced glycosylation of proteins and methods of use therefor.**

(57) The present invention relates to compositions and methods for inhibiting nonenzymatic cross-linking (protein aging). Accordingly, a composition is disclosed which comprises an agent capable of inhibiting the formation of advanced glycosylation end-products of target proteins by reacting with a carbonyl moiety of an early glycosylation product of such target proteins resulting from their initial glycosylation. The method comprises contacting the target protein with the composition. Both industrial and therapeutic applications for the invention are envisioned, as food spoilage and animal protein aging can be treated.

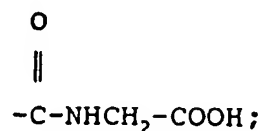
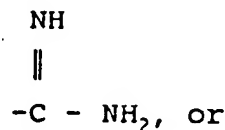
The agents comprise compounds having the following structural formula:



wherein  $R_1$  is  $-CN$ , or

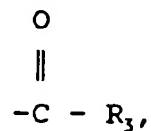


$R_2$  is the same as  $R_1$ , or additionally can be  $-SO_2R$ ,



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R is a lower alkyl group;  
 $R_3$  is R, -OH, -OR, -NH<sub>2</sub>, -NHNH<sub>2</sub>, -NHR;  
 with the proviso that when  $R_1$  and  $R_2$  are independently selected from



wherein  $R_3$  is R, -OR or -NHR, then the lower alkyl portion may be an alkanediyl bridge of 1-5 carbon atoms joining the molecule into a cyclic structure; their pharmaceutically acceptable acid addition salts; and  
 a carrier therefor.



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which under Rule 45 of the European Patent Convention  
shall be considered, for the purposes of subsequent  
proceedings, as the European search report

Application Number

EP 91 10 5259

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
A	EP-A-0 316 852 (THE ROCKEFELLER UNIVERSITY) * The whole document * ---	1-43	A 61 K 31/00 A 61 K 31/12 A 61 K 31/16
A	EP-A-0 222 313 (THE ROCKEFELLER UNIVERSITY) * The whole document * ---	1-43	A 61 K 31/19 A 61 K 31/22 A 61 K 31/275 A 61 K 31/365
A	ANALYTICAL BIOCHEMISTRY, vol. 163, no. 1, 1987, pages 170-174, Academic Press, Inc.; R.S. CROWTHER et al.: "Fluorometric assay of O-linked glycoproteins by reaction with 2-cyanoacetamide" * The whole article * ---	1-2, 5, 22, 23, 26	
			TECHNICAL FIELDS SEARCHED (Int. Cl.5)
			A 61 K
INCOMPLETE SEARCH			
<p>The Search Division considers that the present European patent application does not comply with the provisions of the European Patent Convention to such an extent that it is not possible to carry out a meaningful search into the state of the art on the basis of some of the claims</p> <p>Claims searched completely : Claims searched incompletely : Claims not searched : Reason for the limitation of the search:</p> <p>Remark : Although claims 22-43 are directed to a method of treatment of the human or animal body (Article 52(4) EPC) the search has been carried out and based on the alleged effects of the compositions.</p>			
Place of search THE HAGUE		Date of completion of the search 31-01-1992	Examiner GAC G.
CATEGORY OF CITED DOCUMENTS			
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ..... & : member of the same patent family, corresponding document	

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Application Number

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DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
A	J. POLYMER SCIENCE, vol. 15, no. 11, 1977, pages 2721-2730, John Wiley & Sons, Inc.; D.R. BURFIELD et al.: "Nonoxidative crosslinking reactions in natural rubber. II. Radiotracer study of the reactions of amino acids in rubber latex" * The whole article *	1-2,17-19,22,23,38-40	
A	ANALYTICAL BIOCHEMISTRY, vol. 117, no. 1, 1981, pages 427-432, Academic Press, Inc.; P.M. GALLOP et al.: "Chemical quantitation of hemoglobin glycosylation: Fluorometric detection of formaldehyde released upon periodate oxidation of glycoglobin" * The whole article *	1-2,22,23	TECHNICAL FIELDS SEARCHED (Int. Cl.5)
A	BIOENGINEERING AND THE SKIN, vol. 4, no. 1, January 1988, pages 23-30, Kluwer Academic Publishers, Dordrecht, NL; A. LATA et al.: "Topical bioavailability of beta-aminopropionitrile" * The whole article *	1-3,22-24	

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In view of the large number of compounds which are defined by the general formula of claim 1, the search was limited to the compounds mentioned in claims 4-7,9-10,12-13,15-16,19-21.

